

nanofabrication using the conjugates, and nanomaterials and nanostructures comprising the conjugates. "Recognition oligonucleotides" are oligonucleotides which comprise a sequence complementary to at least a portion of the sequence of a nucleic acid or oligonucleotide target. "Diluent oligonucleotides" may have any sequence which does not interfere with the ability of the recognition oligonucleotides to be bound to the nanoparticles or to bind to their targets.

The invention provides yet another method of binding oligonucleotides to nanoparticles to produce nanoparticle-oligonucleotide conjugates. The method comprises providing oligonucleotides, the oligonucleotides comprising at least one type of recognition oligonucleotides. The recognition oligonucleotides comprise a recognition portion and a spacer portion. The recognition portion of the recognition oligonucleotides has a sequence complementary to at least one portion of the sequence of a nucleic acid or oligonucleotide target. The spacer portion of the recognition oligonucleotide is designed so that it can bind to the nanoparticles. As a result of the binding of the spacer portion of the recognition oligonucleotide to the nanoparticles, the recognition portion is spaced away from the surface of the nanoparticles and is more accessible for hybridization with its target. To make the conjugates, the oligonucleotides, including the recognition oligonucleotides, and the nanoparticles are contacted under conditions effective allow at least some of the recognition oligonucleotides to bind to the nanoparticles. The invention also includes the nanoparticle-oligonucleotide conjugates produced by this method, methods of using the conjugates to detect and separate nucleic acids, kits comprising the conjugates, methods of nanofabrication using the conjugates, and nanomaterials and nanostructures comprising the conjugates.

As used herein, a "type of oligonucleotides" refers to a plurality of oligonucleotide molecules having the same sequence. A "type of" nanoparticles, conjugates, particles, latex microspheres, etc. having oligonucleotides attached thereto refers to a plurality of that item having the same type(s) of oligonucleotides attached to them. "Nanoparticles having oligonucleotides attached thereto" are also sometimes referred to as "nanoparticle-

oligonucleotide conjugates" or, in the case of the detection methods of the invention, "nanoparticle-oligonucleotide probes," "nanoparticle probes," or just "probes."

BRIEF DESCRIPTION OF THE DRAWINGS

5 Figure 1: Schematic diagram illustrating the formation of nanoparticle aggregates by combining nanoparticles having complementary oligonucleotides attached to them, the nanoparticles being held together in the aggregates as a result of the hybridization of the complementary oligonucleotides. X represents any covalent anchor (such as -S(CH₂)₃OP(O)(O⁻)-, where S is joined to a gold nanoparticle). For the sake of simplicity in
10 Figure 1 and some subsequent figures, only one oligonucleotide is shown to be attached to each particle but, in fact, each particle has several oligonucleotides attached to it. Also, it is important to note that in Figure 1 and subsequent figures, the relative sizes of the gold nanoparticles and the oligonucleotides are not drawn to scale.

15 Figure 2: Schematic diagram illustrating a system for detecting nucleic acid using nanoparticles having oligonucleotides attached thereto. The oligonucleotides on the two nanoparticles have sequences complementary to two different portions of the single-stranded DNA shown. As a consequence, they hybridize to the DNA producing detectable changes (forming aggregates and producing a color change).

20 Figure 3: Schematic diagram of a variation of the system shown in Figure 2. The oligonucleotides on the two nanoparticles have sequences complementary to two different portions of the single-stranded DNA shown which are separated by a third portion which is not complementary to the oligonucleotides on the nanoparticles. Also shown is an optional filler oligonucleotide which can be used to hybridize with the noncomplementary portion of the single-stranded DNA. When the DNA, nanoparticles and filler oligonucleotides are combined, the nanoparticles aggregate, with the formation of nicked, double-stranded
25 oligonucleotide connectors.

Figure 4: Schematic diagram illustrating reversible aggregation of nanoparticles having oligonucleotides attached thereto as a result of hybridization and de-hybridization

with a linking oligonucleotide. The illustrated linking oligonucleotide is a double-stranded DNA having overhanging termini (sticky ends) which are complementary to the oligonucleotides attached to the nanoparticles.

5 **Figure 5:** Schematic diagram illustrating the formation of nanoparticle aggregates by combining nanoparticles having oligonucleotides attached thereto with linking oligonucleotides having sequences complementary to the oligonucleotides attached to the nanoparticles.

10 **Figure 6:** Cuvettes containing two types of gold colloids, each having a different oligonucleotide attached thereto and a linking double-stranded oligonucleotide with sticky ends complementary to the oligonucleotides attached to the nanoparticles (see Figure 4). Cuvette A - at 80°C, which is above the Tm of the linking DNA; de-hybridized (thermally denatured). The color is dark red. Cuvette B - after cooling to room temperature, which is below the Tm of the linking DNA; hybridization has taken place, and the nanoparticles have aggregated, but the aggregates have not precipitated. The color is purple. Cuvette C - after several hours at room temperature, the aggregated nanoparticles have settled to the bottom of the cuvette. The solution is clear, and the precipitate is pinkish gray. Heating B or C will result in A.

15 **Figure 7:** A graph of absorbance versus wavelength in nm showing changes in absorbance when gold nanoparticles having oligonucleotides attached thereto aggregate due to hybridization with linking oligonucleotides upon lowering of the temperature, as illustrated in Figure 4.

20 **Figures 8A-B:** Figure 8A is a graph of change in absorbance versus temperature/time for the system illustrated in Figure 4. At low temperatures, gold nanoparticles having oligonucleotides attached thereto aggregate due to hybridization with linking oligonucleotides (see Figure 4). At high temperature (80°C), the nanoparticles are de-hybridized. Changing the temperature over time shows that this is a reversible process. Figure 8B is a graph of change in absorbance versus temperature/time performed in the same